c. minimally replating with an inhibitor to optimize the dopaminergic phenotype and a purified harvest[; and

d. harvesting the dopaminergic, differentiated neuronal cells].

11. (amended) A dopaminergic neuronal cell [suitable for transplantation into an individual having a dopaminergic deficiency], said cell comprising

a post-mitotic differentiated neuronal cell which expresses tyrosine hydroxylase and at least one other indicator of neuronal cell differentiation, said cell having undergone induction from an undifferentiated cell.

12. (amended) A human post-mitotic dopaminergic cell [suitable for transplantation into a human having a dopaminergic deficiency], said cell comprising a differentiated neuronal cell which expresses tyrosine hydroxylase and at least one other indicator of neuronal cell differentiation, said cell having undergone induction from an undifferentiated human cell.

13. (amended) A human dopaminergic cell [suitable for transplantation into a human having a dopaminergic deficiency], the cell comprising a differentiated human neuronal cell that expresses tyrosine hydroxylase and bcl-2, said cell being capable of synthesizing dopamine and having improved survival [after transplantation].

14. (amended) A method of improving the survival of human neuronal cells [for transplantation], said method comprising the steps of

a. providing a culture of human <u>neuronal</u> cells; <u>and</u>

b. adding a lithium salt to the human <u>neuronal</u> cell culture for a sufficient time to enhance expression of bcl-2[;

c. testing cells from the treated cell culture for the presence of bcl-2;

d. isolating the cells from the culture to produce an isolated cell preparation; and

e. testing the isolated cell preparation for sterility before packaging the cells for transport].

15. (amended) A pharmaceutical dosage form of human non-fetal dopaminergic cells [suitable for transplantation in Parkinson's Disease] comprising

isolated, [purified] neuronal cells, the neuronal cells <u>being capable of</u> expressing tyrosine hydroxylase, D2 dopamine receptor and aldehyde dehydrogenase-2; and

a pharmaceutical diluent.

17. (amended) The method of claim <u>14</u> [15] wherein the lithium salt is lithium chloride.

19. (amended) A method of preparing human dopaminergic neuronal cells [suitable for treating Parkinson's disease], the method comprising:

a. providing NT2/D1 cells;

b. culturing NT2/D1 cells with an inducing agent for a time sufficient to optimize tyrosine hydroxylase (TH) expression therein; and

c. replating and culturing the TH-optimized cells in mitotic inhibitor[; and

d. separating the TH-optimized cells from the replate culture].

20. The method of claim 19, additionally comprising the steps of

e. replating the TH-optimized cells on a confluent feeder cell layer, the cell layer being chosen from cells which stabilize[d] TH production [including bone marrow stem cells, TM4 Sertoli cells, glioma cells, or a combination thereof]; and

f. isolating the TH-optimized and stabilized cells from the replate medium.

21. (amended) A pharmaceutical composition [for treating Parkinson's Disease, the composition] comprising

isolated, [purified,] post-mitotic neuronal cells, the neuronal cells expressing tyrosine hydroxylase (TH), D2 dopamine receptor, and aldehyde dehydrogenase-2;

cells capable of stabilizing TH [tyrosine hydroxylase] production of the neuronal cells; and

a pharmaceutical diluent.

23. (new) The method of claim 20 wherein the cells which stabilize TH production comprise bone marrow stem cells, TM4 Sertoli cells, glioma cells, or a combination thereof.

Response

The claimed invention comprises a way to induce progenitor cells to become dopaminergic (DA) cells, that is, to produce dopamine. The way this is done is through adding chemicals to the cells which cause the cells to produce different proteins. In one embodiment, the claimed method starts with NT2/D1 cells that lack a protein which is an indicator of neuronal cell differentiation. The NT2/D1 cells are treated with retinoic acid, an agent that causes some of the cells to express tyrosine hydroxylase, as indicated in Figure 1 of the patent application. Other cells do not change and are called accessory cells. Then the induced cell culture is exposed to inhibitors cytosine arabinoside and fluorodeoxyuridine for several days, during which the DA cells continue to mature and the accessory cells stop dividing and die off. Then the DA cells are harvested. The claimed method does not genetically alter or transform the cells.

The DA cells also have the other cellular attributes necessary to regulate dopamine. Specifically the D2 dopamine receptor (D2) regulates dopamine release, the dopamine membrane transporter (DAT) is responsible for reuptake of dopamine from the synaptic cleft, and aldehyde dehydrogenase (AHD2) has been found in a subpopulation of DA neurons found in the mesostriatal and mesolimbic areas, which also are involved in Parkinson's disease. Table 1 of the specification (page 18) shows that all three proteins plus tyrosine hydroxylase are present in the DA neurons.

The specification goes on to exemplify the use of the DA neurons in mammalian research. Example 12 (pages 28-32) shows the successful use of DA neurons in the rat model of Parkinson's disease. Example 12 provides details on exactly how to use the DA neurons in this use, including exact neurosurgical coordinates for cell placement and specific dosages. The results indicate that the DA neurons were indeed implanted in the desired locations and that dopamine-producing cells could still be detected six-weeks post transplant. The DA-implanted rats also had somewhat improved rotational scores, an indication of functional improvement.

Section 112, paragraph 1 Rejection

The Office Action stated that claims 1-17 and 19-22 stand rejected because the specification did not enable one skilled in the art to make and/or use the claimed invention. Initially the claims all recited use in transplantation. The claims have been amended to delete the use in transplantation. The claims now are focused on methods for producing the DA cells and the DA cells themselves, which as summarized above are capable, dopaminergic neurons.

The Office Action stated that methods of transplantation of neural tissue are not routinely successful; however, the patent application has a working example (Example 12) that shows the successful use of the DA neurons in rats. Applicants respectfully observe that Example 12 does teach the number of cells to inject, the site of injection and cellular persistence, as required by the Examiner. Therefore, this ground for rejection may be withdrawn.

The Office Action also noted that the specification teaches that the only use for the method is to produce cells for gene therapy. As summarized above, the claimed cells are not genetically transformed, they are merely induced by the presence of various chemicals to increase production of dopaminergic factors. Moreover, the specification states: "we demonstrate in the studies disclosed herein that the phenotype of the NT2/D1 cells can be altered by certain culture conditions – without transfection of foreign genes – to consistently produce the levels of DA to ameliorate abnormal dopaminergic conditions. (page 9, lines 11-13)" Since the claimed invention does not include genetic modification, this rejection on the nonenablement of gene therapy may be withdrawn.

Conclusion

Applicants believe that all grounds for rejection have been overcome and a notice of allowance can be issued. To resolve any remaining issues, the Examiner is cordially invited to telephone the undersigned.

Respectfully submitted,
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